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Some Selected Serological Diagnostic Techniques in Plant Virology

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1. Introduction

Particle morphology, host range and the serological properties of the coat protein are generally being used to identify plant viruses. In classification and the establishment of taxonomic relationships, cross-reactivity of antisera raised against viruses from different groups have been frequently used (KPL Technical Guide on line 1999/2000 edition). However sequence data of nucleic acid are accumulating rapidly and are allowing more accurate relationships to be established between the individual members of virus groups than serological methods do (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004). Sequencing parts of a virus genome is often done in the identification of a virus if detailed serological analysis cannot provide conclusive data about the virus (Dellaporta *et al.* 1983). This involves purification and isolation of the virus particles and the subsequent cloning of parts of the virus. Using advance molecular methods in specific Polymerase chain reaction (PCR), new virus sequence data can be obtained without the need to purify a virus or to clone parts of its genome (Jackie Hughes *et al* 2004). Advances in molecular biotechnology have contributed immensely in developing specific and sensitive diagnostic assays and sensitive antisera against individual viruses or group of viruses (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004). Useful tools are provided in diagnostic procedures for virus disease monitoring.

2. Virus diagnosis

Different diagnostic techniques are used in the identification of plant viruses. Symptom observations is an important tool in field virus diagnosis though it is not a conclusive methods, it is combined with other methods (Fajinmi, 2006). One of such methods is transmission studies, which involves studies on how a virus gets into host plants. Different indicator test plants are mechanically inoculated or graft transmitted with the virus, insect vector transmission of the virus are carried out on series of test plants in specialized insect proof cages so as to provide useful information and further characterizing the virus (Jackie Hughes *et al* 2004; Fajinmi, 2011). Environmental factors may influence the symptom expression of the virus which may serve as a limiting factor but the use of electron microscopy brings further clarification of the virus (Jackie Hughes *et al* 2004; Fajinmi, 2006; Fajinmi, 2011).

3. Types of serological tests

There are principally two types of serological test, the liquid phase test and the solid phase test (Jackie Hughes *et al* 2004). Both methods are basically interaction between known antibodies and the unknown virus for positive identification, but visualization differs. In liquid phase test, there is formation of a visible precipitate in a solution reaction, i.e. agglutination of visible cells. This kind of test include, gel diffusion test, precipitin or micro-precipitin test (Jackie Hughes *et al* 2004).

In solid phase test, it is an enzymatic reaction, where antibodies are conjugated with a marker enzyme in which the antibody attaches itself with the antigen after recognizing it (Jackie Hughes *et al* 2004). The chosen enzyme reacts with the substrate to provide a positive identification. This is confirmed through colour change in the substrate. Examples of this test include micro-titre plates or nitrocellulose membranes (Jackie Hughes *et al* 2004).

4. Test methods selection

Test method could be selected depending on the reason of the serological analysis, the information required and availability of materials to be used (Jackie Hughes *et al* 2004). Diagnosis is performed in optimum conditions with proper controls and must be reproducible. This will enhance proper, correct and desirable data analysis for the result to be valid. Availability of laboratory equipment and antibodies will determine the specific serological test to be used (Jackie Hughes *et al* 2004).

5. Serological techniques

5.1 Double diffusion tests

The materials needed to design, perform and evaluate double diffusion tests includes, (1) Pipette (10 μ l – 100 μ l is preferred; other pipettes can be used) (2) Flat bottomed glass or plastic dishes (Petri dishes are recommended) (3) Agar (4) phosphate Buffer (5) cork borer for punching out well patterns (6) tight sealed moist chamber for incubation with moist towel in bottom (Jackie Hughes *et al* 2004).

Test Procedure as described by Jackie Hughes *et al* (2004)

- a. Dissolve 70mg of agar in 100mls of buffer (0.7 % W/V) and autoclave at 120°C for 10 minutes, Cool to 60°C.
- b. Dispense to sterilized Petri-dishes and allow to fully solidify.
- c. With the aid of a cork borer, punch out well patterns on the solidified agar in the Petri-dish with no damage to the clean – cut sides of the wells.
- d. Dispense the antibodies and antigen to the wells according to the test pattern.
- e. Incubate test plate in the moist chamber for 5-7 days.

6. Determination of optimum antigen/antibody concentrations

Optimum concentration for antigen and antibodies can be determined by creating a pattern of six wells in a circular pattern around one central well (Jackie Hughes *et al* 2004). The six wells are filled with different concentration of antibodies (double fold dilutions mg/ml). In

another plate different concentration of antigen could be used (Jackie Hughes *et al* 2004). A thin sharp line of precipitation will be shown between the antigen well and the antibody well that is having the right concentration (Jackie Hughes *et al* 2004).

7. Interpreting double diffusion test results

There is a radial diffusion of the antigen and antibodies from the wells. Lines of precipitate are formed in the agar as the antibodies and antigen meet; results are interpreted based on these patterns of precipitate (Jackie Hughes *et al* 2004). Combination of antibodies and antigen must be properly balanced with the correct dilutions for correct interpretation (Jackie Hughes *et al* 2004).

8. Enzyme-Linked Immunosorbent Assay (ELISA) tests

This is the most common technique for diagnosing of plant viruses and it is a solid phase assay. The test reaction involves antibody and antigen reaction to produce a positive reaction. The reaction takes place in a microtitre plates made of either polystyrene or polyvinyl chloride (PVC) (KPL Technical Guide (on line 1999/2000 edition); Jackie Hughes *et al* (2004). Antibodies conjugated to a “marker enzyme” are used in ELISA. Reaction between the antibody and the antigen will make the “marker enzyme” to react in the substrate resulting in colour development (KPL Technical Guide on line 1999/2000 edition); Jackie Hughes *et al* 2004).

There is “direct” and “indirect” ELISA test. In “direct” ELISA test, the detecting antibody bounds with the marker enzyme while in “indirect” ELISA test, the antibody enzyme conjugate binds with the detecting primary antibody and not directly with the virus (KPL Technical Guide on line 1999/2000 edition); Jackie Hughes *et al*. 2004).

“Direct” ELISA procedure as described by KPL technical guide (on line 1999/2000 edition) and Jackie Hughes *et al* (2004)

Coat the microtitre plate with antibodies followed by the virus sample. The virus becomes bound to the antibodies for a positive reaction. Wash the plates with phosphate buffer in Tween-20 to remove any of the virus samples that has not reacted with the antibodies. Add antibody enzyme conjugates then wash again to remove excess unbound conjugate then add the substrate. Colour development provides an indication that the virus and the antibody have reacted. This shows that the antibody enzyme conjugates has attached to the trapped virus allowing the enzyme to react. This confirms a tentative virus identification and quantification. This test method is referred to as double antibody sandwich (DAS) ELISA because the virus is sandwiched between the capturing antibody and the detecting antibody. This method is strain specific and the antibody to detect the virus must be conjugated to an enzyme and a specific conjugate is required for each antibody to be used.

“Indirect” ELISA

This involves the usage of antibodies which have been raised to two different animals and using different methods for capturing the virus (KPL Technical Guide on line 1999/2000

edition). Though the antibodies that identify with the virus have been raised for that specific virus, the secondary antibody marker can be raised to recognize a wide range of primary antibodies, with the fact that the primary antibodies were produced in the same animal species against which the secondary antibodies were raised (Jackie Hughes *et al* 2004). It is cheaper to produce a secondary marker antibody enzyme conjugate in “indirect” ELISA than primary marker antibody enzyme conjugate in “direct” ELISA (Jackie Hughes *et al* 2004). Two different animals are used in the production of primary and secondary antibodies (Jackie Hughes *et al* 2004). In recognizing specific viruses, primary antibodies are raised in one animal species, while production of secondary antibodies is to recognize proteins from specific species of animal (Jackie Hughes *et al* 2004). For instance, antibodies as protein source from rabbit can be used as antigen to produce secondary antibody from mouse (Jackie Hughes *et al* 2004). These secondary antibodies from mouse can bind with any antibodies produced in a rabbit.

Examples of “indirect” ELISA methods as described by KPL Technical Guide (on line 1999/2000 edition) and Jackie Hughes *et al* (2004) include; Antigen coated plate (ACP) or plate trapped (PTA), direct antigen coated (DAC) assays. In this assays, the first to be added is the primary antibodies, then the secondary antibody enzyme conjugate and substrate. In triple antibody sandwich (TAS) ELISA, three antibodies are used in a sequential order. Trapping antibodies followed by the virus sample then the primary antibodies added followed by the secondary enzyme conjugated antibodies which binds with the primary antibodies. Protein A sandwich (PAS) ELISA uses protein A for an increase in the test specific and reaction sensitivity by controlling orientation of antibodies. Steps involve; Plate covered with protein A → trapping antibodies → virus sample → secondary antibody. Protein A will detect by binding with the secondary antibodies if in correct orientation.

9. ELISA buffers

9.1 Phosphate buffered saline

8.0 g NaCl
0.2 g KH_2PO_4
1.1 g Na_2HPO_4
0.2 g KCL

(Add up to litre with distilled water)

9.2 PBS-tween

Add 0.5ml Tween 20 (0.05%v/v) to 999.5 ml PBS pH 7.4

9.3 Coating buffer pH 9.6

1.59 g Na_2CO_3
2.93 g NaHCO_3

(Add up to litre with distilled water)

9.4 Conjugate buffer

Half strength PBS containing
0.05% v/v Tween 20
0.02% w/v egg albumin (ovalbumen)
0.2% w/v polyvinylpyrrolidone (PVP)

9.5 Substrate buffer pH 9.8

97 ml diethanolamine
800 ml H₂O
Add HCL to give pH 9.8

(Add up to litre with distilled water)

Store all buffers at 4°C and monitor its pH before use. Buffers should be replaced if not used within one month.

Examples of ELISA Virus indexing protocols as described by KPL Technical Guide (on line 1999/2000 edition) and Jackie Hughes *et al* (2004)

Weigh 1 g of leaf sample in 3 ml of extraction buffer (PBS-Tween + 2% (w/v) Polyvinylpyrrolidone (PVP) in a sterilized mortar and grind with a sterile pestle in a circular motion to form a paste. Sieve supernatant by carefully removing the plant debris into sterile Eppendorf tube container.

Example protocols for Antigen Coated Plate (ACP) ELISA as described by KPL Technical Guide (on line 1999/2000 edition) and Jackie Hughes *et al* (2004).

1. Add 100µl of antigen ground in extraction buffer into plates
2. Cover plate and incubate over night at 4°C
3. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
4. Block with 200µl per well of 3% (w/v) dried non fat skimmed milk in PBS-Tween
5. Cover plate and incubate at 37°C for 30 minutes
6. Empty and tap plate dry
7. Add 100µl per well of antibody (monoclonal or polyclonal) diluted in conjugate buffer 1:2000
8. Cover plate and incubate at 37°C for 2 hours
9. Wash plate 3 times with PBS-Tween by flooding for 3 times. Tap plate dry
10. Add 100µl per well of either goat anti mouse or goat anti-rabbit alkaline phosphate conjugate diluted in conjugate buffer 1:2000
11. Cover plate and incubate at 37°C for 2 hours
12. Wash plate 3 times with PBS-Tween by flooding for 3 times. Tap plate dry
13. Add 200µl per well of 0.5- 1 mg/ml of p-nitrophenyl phosphate substrate in substrate buffer.
14. Read the plate after 1 hour and or overnight.

Example protocols for Protein – A sandwich (PAS) ELISA as described by KPL Technical Guide (on line 1999/2000 edition) and Jackie Hughes *et al* (2004).

1. Add 100µl of protein A per well in extraction buffer into plates

2. Cover plate and incubate the plates at 37°C for 2 hours
3. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
4. Grind virus free leaf sample in PBS-Tween at a dilution of 1:5
5. Dilute the antiserum 1: 2000 in virusfree leaf extract and incubate for 20-30 minutes at 37°C to cross-adsorbed any antibodies to plant proteins.
6. Add 100µl per well of cross-adsorbed polyclonal antiserum to each well
7. Cover plate and incubate at 37°C for 2 hours
8. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
9. Add 100 µl of antigen (virus sample) ground in PBS-Tween + 2% PVP
10. Cover plate and incubate overnight at 4°C
11. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
12. Add 100 µl of diluted (as in step 5) and cross-adsorbed polyclonal antiserum
13. Cover plate and incubate at 37°C for 2 hours
14. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
15. Add 100 µl per well of Protein A Alkaline phosphate conjugate at a dilution of 1:1000 in conjugate buffer
16. Cover plate and incubate at 37°C for 2 hours
17. Wash plate 3 times with PBS-Tween by flooding 3 times. Drain and tap plate dry
18. Add 200 µl per well of 0.5-1 mg/ml p-nitrophenyl phosphate substrate in substrate buffer
19. Read the plate after 1 hour and or overnight

10. Positive and negative controls

For every ELISA test there are positive and negative controls which must be from the same genotype (s) as the samples. These are used to determine if the samples are infected. Two positive control samples well derived from plant infected with the virus to be diagnosed should be included in every test. Samples material used for negative control must come from plant that has tested negative for virus infection (KPL Technical Guide on line 1999/2000 edition; Jackie Hughes *et al* 2004).

11. ELISA plate layout

Determination of plate layout varies with the test to be carried out. New clean ELISA plates should be used for new test to avoid contamination. To avoid edge effect due to inconsistency of the results to be obtained, the outside wells should not be used for any test. Positive control wells are advised to be put at the top left hand corner while the negative control wells are put at the bottom right hand corner (KPL Technical Guide on line 1999/2000 edition; Jackie Hughes *et al* 2004). The remaining wells then serves as test sample wells using two wells per sample test. Non water soluble marker pens should be used to demarcate various test sample areas (Jackie Hughes *et al* (2004).

12. ELISA result interpretation

There are two basic type of ELISA reader. One reads the entire plate at once while the other reads individual well if correctly used. Plate's bottom to be read must be clean and dry and result read at room temperature. Contact the user's manual for the ELISA reader for further

assistance. ELISA result is read using nanometer reading set at A_{405} infection ($A_{405 \text{ inf}}$) no matter the type of ELISA reader used (Jackie Hughes *et al* (2004). A sample test is considered positive for the virus if its ELISA reading doubles that of the negative control (KPL Technical Guide on line 1999/2000 edition). In some cases the sample test is considered virus positive if the ELISA reading is 1.5 times the means of the negative controls (Jackie Hughes *et al* (2004);

13. Polymerase Chain Reaction (PCR)

This is a method for nucleic acid amplification *in-vitro*. The product of the polymerase chain reaction is been used by PCR for identification. It is highly sensitive; apart from identifying the DNA it also amplifies the target nucleic acid sequence (Dellaporta *et al.* 1983). It uses multiple cycles of template denaturation, primer annealing and primer elongation to amplify DNA sequence (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004). Each step occurring at specific temperature for specified time period.

Components of PCR as described by Dellaporta *et al.* (1983) and Jackie Hughes *et al* (2004)

Template: Nucleic acid of infected plant or virus DNA. RNA viruses are converted to cDNA before amplification.

Primers: Sequences of short fragment of oligonucleotides (single stranded DNA) complementary to the sequences at the end of target sequences to be amplified. Forward and reverse primers are required in this reaction.

dNTPs: Nucleotides required for the extension of the newly synthesized DNA strand. dATP, dCTP, dGTP, dTTP (the four nucleotides) are included in the mixture

Enzyme: A thermostable (heat resistant) enzyme is required for polymerization due that the polymerase reaction undergoes denaturation of template at high temperature. Taq polymerase is the thermostable polymerase enzyme used frequently.

13.1 Reaction buffer and magnesium chloride

The magnesium ion concentration affects among others, enzyme activity, primer annealing and denaturation (Jackie Hughes *et al* 2004).

PCR reaction is carried out in a thermo cycler that has been programmed to cycle between high and low temperatures within a specified period of time. This involves 3 main steps as described by Jackie Hughes *et al* (2004).

1. Template DNA denaturation
2. Annealing of primers
3. Extension of DNA strand

Two new DNA strands as explained by Jackie Hughes *et al* (2004) are formed at the end of the first cycle totaling four strands of DNA template which serves as templates for the second cycle. In the second cycle, the new DNA elongates as the primers binds to the templates. A total of eight strands would have been synthesized at the end of the second cycle. The DNA increases at exponential rate with each cycle as the PCR continues until set

number of cycles has been reached. Ethidium bromide stained gel is used to visualize the final product (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004).

PCR Reaction mixture as described by Dellaporta *et al.* (1983) and Jackie Hughes *et al* (2004)

To detect individual viruses, specific primers are to be used.

Reagent	Stock concentration	Working concentration
Buffer	10x	1x
MgCl	25 mM	1.5 mM
dNTPs	20 mM	
Primer 1		10-100 pmole
Primer 2		10-100 pmole
Taq polymerase	5 units/ µl	0.8-2.5 units
DNA		1-10 ng

13.2 Reverse Transcriptase PCR (RT-PCR)

Viruses with RNA are amplified with reverse transcriptase as their nucleic acid in PCR. DNA is used as template for Taq polymerase; therefore the virus will have to convert its RNA to cDNA before the polymerase reaction. Short cDNA are formed due to cleavages caused in the RNA due to the contaminating RNase. One-tube reaction or two-tube reactions can be used to carry out RT-PCR (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004).

13.3 Two-tube RT-PCR

RNA extracted from the virus as template or total nucleic acid extracted from the infected plant is used to synthesize cDNA by reverse transcription. An aliquot of the reverse transcription reaction is used in the PCR. Usually, not more than 1/5 of the total PCR mixture should derived from the reverse transcription reaction (Jackie Hughes *et al* 2004).

13.4 One-tube RT-PCR

This is where the same buffer is used to carry out the reverse-transcription reaction and PCR so the two reactions can not be separately optimized. Before adding the mixture, the RNA need to be denatured at 70-75°C for 5 min so that there can be good synthesis of cDNA which is immediately followed with a PCR cycling programme (Jackie Hughes *et al* 2004). The thermocycler can accommodate both programmes at the same time as one programme (Jackie Hughes *et al* 2004).

13.5 Immunocapture PCR

Along with the PCR the virus particle could be trapped using the antisera. This reaction could be carried out in a single tube. This method is good for viruses having low concentration in the plant or viruses having their genome integrated into host plant genome (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004).

13.6 Immunocapture reverse transcription PCR

In this test RNA is used as the template which makes it differs from immuno-capture PCR which make it very efficient in detecting RNA in viruses from plant that could have affected the enzymes in the reverse transcription reaction or the PCR through some inhibitory compounds that it contains (Jackie Hughes *et al* 2004). A single tube can be used to carry out immuno-capture, reverse transcription and the PCR and the different reactions could be carried out separately.

An example of a typical IC-RT-PCR as described by Jackie Hughes *et al* (2004).

1. Coat tubes with polyclonal antibodies diluted in coating buffer (200µl)
2. Incubate at 37°C for 2-3 hours
3. Wash tubes with PBS-Tween
4. Add 200µl of sample ground in (ELISA) sample buffer
5. Incubate overnight at 4°C or 2-3 hr at 37°C
6. Wash two times with PBS-Tween
7. Add RT-PCR mix and subject to thermal cycling consisting of one cycle of RT

RT-PCR Mix

10x PCR buffer	1x
MgCl	2.0 mM
dNTPs mix	0.5 mM
Primers	10 pmole each
Taq DNA polymerase	2.5 units
Reverse Trancriptase	200 units
RNase free water	–
Total volume	25-50 µl

Cycling condition should consist of an initial one cycle for the reverse transcription linked to the PCR cycle.

14. Extraction of DNA for PCR

The DNA extraction procedure as developed by Jackie Hughes *et al* (2004).

1. Grind about 50mg of young infected plant material in 500 µl of extraction buffer
2. Pour sap into a new and sterilized Eppendorf tube.
3. Add 33ul of 20% sodium dodecyl sulphate (sds)
4. Vortex briefly and incubate in water bath at 65°C for 10 min
5. Bring to room temperature and add 160ul of 5M potassium acetate
6. Vortex and centrifuge at 10,000 g for 10 min
7. Remove the supernatant (about 400 µl) into another Eppendorf tube
8. Add 200 µl of cold iso-propanol
9. Mix gently and keep on ice or at 4°C for 15-20 min
10. Centrifuge at 10,000 g for 10 min to sediment the DNA
11. Decant the supernatant gently and ensure that the pellet is not disturbed
12. Add 500 µl of 70% ethanol to the pellet (this is to wash it) and centrifuge at 10,000g for 5-10 min.

13. Decant the ethanol and air-dry the DNA (at room temp) until no trace of alcohol can be seen in the tube.

The DNA is re-suspended in 950 µl TE or sterile distilled water) and stored in the freezer as stock solution. The DNA is usually diluted 1×10^5 times for PCR, but it will be better to do a dilution curve to determine the best dilution for amplification.

Extraction buffer	TE buffer
100 mM (pH 8.0)	10 mM Tris
8.5 mM EDTA	1 mM EDTA
500 mM NaCl	
10 mM β -mercaptoethanol (added just before use)	

Extraction of total nucleic acids for RT-PCR (Method A) as described by Jackie Hughes *et al* (2004)

1. Grind 50-100 mg of young leaf sample in 500 µl of extraction buffer using cooled sterilized pestle and mortar.
2. Add proteinase K and SDS to a final concentration of 50 mg/ml and 0.1% respectively.
3. Incubate the mixture at 37°C for 5 min
4. Add 500 µl of phenol and vortex for a few seconds.
5. Centrifuge at 5,000g for 5 min.
6. Remove the aqueous phase into another Eppendorf tube
7. Add 35 µl of chloroform and vortex briefly
8. Remove the aqueous phase and repeat the chloroform extraction..
9. Remove the aqueous phase into another Eppendorf tube and add 1/10 volume of 3 M sodium acetate and twice the volume of cold ethanol and precipitate nucleic acid overnight at -20°C.
10. Spin at 10,000g for 15 min and pour off the ethanol.
11. Wash nucleic acid with 70% ethanol.
12. Dry nucleic acid at room temperature.
13. Re-suspend nucleic acid in 20 µl of sterile distilled water.

14.1 Extraction buffer

50 mM Tris-HCl pH 8.0
 10 mM EDTA
 1 mM DTT
 50 mM NaCl

Extraction of total nucleic acid for PCR (Method B) as described by Jackie Hughes *et al* (2004).

1. Grind 50-100 mg leaf sample in liquid nitrogen.
2. Add 600 µl of CTAB buffer and mix thoroughly.
3. Incubate at 55°C for 15-30 min
4. Shake mixture with 300 µl of chloroform; isomyl alcohol (24:1)

5. Centrifuge at 10,000g for 5 min.
6. Remove the aqueous phase into a fresh Eppendorf tube and add tenth volume of 7.5-ammonium acetate or 3 M sodium acetate.
7. Add equal volume of iso-propanol and keep for 15 min at 4°C
8. Centrifuge at 10,000 g for 10 min and wash the pellet with 70% ethanol
9. Dry total nucleic acid at room temperature.

14.2 CTAB buffer

Cetyltrimethyl ammonium bromide	2% (w/v)
NaCl	1.4 M
Tris-HCl (pH 8.0)	0.1M
β-mercaptoethanol	0.5%

15. Electrophoresis of nucleic acids

Nucleic acids can be size fractionated in agarose or polyacrtlamide gels (Dellaporta *et al.* 1983). Agarose gels are frequently used because it is easy to prepare and safer compared to polyacrylamide (Dellaporta *et al.* 1983). The nucleic acid is stained with ethidium bromide that can be incorporated in the gel during preparation or stained after electrophoresis. Nucleic acids migrate towards the positive pole in an electric field in an appropriate buffer (Dellaporta *et al.* 1983; Jackie Hughes *et al* 2004).

15.1 Electrophoresis and gel loading buffer

Tris acetate buffer (TAE) and Tris-orate buffer are the commonly used electrophoresis buffer

Preparation of an Agarose Gel as described by Jackie Hughes *et al* (2004)

1. Seal the ends of the plastic tray supplied with the electrophoresis apparatus with tape.
2. Prepare sufficient electrophoresis buffer to fill the tank and to prepare the gel, measure the quantity of agarose required into an Erlenmeyer flask or glass bottle with a loose-fitting cap and add the buffer to the agarose to give the required concentration of gel (usually 1%w/v is used for most analyses).
3. Heat the agarose suspension in a microwave oven or a heater until it boils and dissolves
4. Cool solution to 60°C and if desired add anthodium bromide (from a stock solution of 10 mg/ml in water) to a final concentration of 0.5 µg/'ml and mix thoroughly.
5. Position the comb in the tank. If the comb is too close to the plate, there is a risk that the base of the well may tear when the comb is withdrawn, allowing the sample to leak between the gel and the plate.
6. Pour the agarose into the prepared tray and allow to set at room temperature.
7. After the gel is completely set (30-45 min) carefully remove the comb and tape and mount the gel in the electrophoresis tank.
8. Add enough electrophoresis buffers to cover the gel to a depth of about 1mm.
9. Mix the sample DNA with the desired gel-loading buffer and slowly load the mixture into the slots of the submerged gel using a disposable micropipette.

10. Attach electrical leads to the tank so that the DNA will migrate toward the anode (usually the red lead). Apply a voltage 1-5 V/cm to the gel. Run the gel until the bromophenol blue has migrated an appropriate distance from the gel.
11. Turn off the electric current and remove the leads. If ethidium bromide is present in the gel, examine the gel by ultraviolet light.

If the anthodium bromide was not added to the gel, the gel must be stained by soaking in a solution containing ethidium bromide (0.5 µg/ml) for about 30-45 min.

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Serological Diagnosis of Certain Human, Animal and Plant Diseases

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This book explains the concept of serological methods used in laboratory diagnoses of certain bacteria, mycoplasmas, viruses in humans, animals and plants, certain parasitic agents as well as autoimmune disease. The authors present up-to-date information concerning the serological methods in laboratory diagnosis of such infectious diseases. Section one deals with the serological methods for bacteria. Section 2 deals with serological methods in human, animal and plant viruses. Section 3 is concerned with the serological laboratory diagnosis of echinococcus and human toxocariasis agents. The last section deals with serological laboratory methods in the diagnosis of coeliac disease.

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